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Interspecies Transduction of Plasmids among *Bacillus anthracis*, *B. cereus*, and *B. thuringiensis*

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Bacteriophage CP-51, a generalized transducing phage for *Bacillus anthracis*, *B. cereus*, and *B. thuringiensis*, mediates transduction of plasmid DNA. *B. cereus* GP7 harbors the 2.8-megadalton multicopy tetracycline resistance plasmid, pBC16. *B. thuringiensis* 4D11A carries pC194, the 1.8-megadalton multicopy chloramphenicol resistance plasmid. When phage CP-51 was propagated on these strains, it transferred the plasmid-encoded antibiotic resistances to the nonvirulent Weybridge (Sterne) strain of *B. anthracis*, to *B. cereus* 569, and to strains of several *B. thuringiensis* subspecies. The frequency of transfer was as high as 10^{-5} transductants per PFU. Tetracycline-resistant and chloramphenicol-resistant transductants contained newly acquired plasmid DNA having the same molecular weight as that contained in the donor strain. Antibiotic-resistant transductants derived from any of the three species were effective donors of plasmids to recipients from all three species.

Procedures for introducing plasmids into new hosts are valuable tools in the study of many microorganisms. Plasmid transduction has been well documented and among *Bacillus* species was first shown in *B. pumilus* (3). The broad-host-range bacteriophage CP-51, isolated from soil by Thorne (7), is active in the generalized transduction of *B. cereus*, *B. anthracis*, and *B. thuringiensis* (7–9). It transduces chromosomal markers at frequencies ranging from 1×10^{-7} to 5×10^{-6} transductants per PFU and presumably can carry up to 60 megadaltons of host DNA corresponding in size to that of the phage genome (13). *B. cereus* GP7 and *B. thuringiensis* 4D11A carry antibiotic resistance plasmids, and both of these strains lie within the host range of the phage. *B. cereus* GP7 harbors pBC16, a 2.8-megadalton multicopy tetracycline resistance plasmid (2), and *B. thuringiensis* 4D11A carries pC194, a 1.8-megadaldon multicopy chloramphenicol resistance plasmid originally isolated from *Staphylococcus aureus* (4). Both plasmids have been extensively studied in species of *Bacillus*. We report here that phage CP-51 is able to transduce these plasmids into and among strains of *B. anthracis*, *B. cereus*, and *B. thuringiensis*.

MATERIALS AND METHODS

Organisms. The organisms used in this study are listed in Table 1.

Bacteriophage. Phage CP-51 and the methods used for its propagation and assay have been described previously (7–10, 12, 13). Phage lysates were routinely treated with DNase (50 μg/ml) to inactivate bacterial DNA, and they were confirmed to be free of bacterial contamination by plating samples on nutrient broth-yeast extract (NBY) agar.

Media and growth conditions. NBY medium contained 8 g of nutrient broth (Difco Laboratories, Detroit, Mich.) and 3 g of yeast extract (Difco) per liter, pH 6.8. L-broth contained 10 g of tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl per liter, pH 7.0. BHI-glycerol medium contained 37 g of brain heart infusion (Difco) and 5 g of glycerol per liter. Min 3C was composed of the following (in grams per liter, with the pH adjusted to 7.0 with NaOH): $(NH_4)_2SO_4$, 2; KH_2PO_4, 6; K_2HPO_4, 14; $MgSO_4 \cdot 7H_2O$, 0.2; $FeCl_3 \cdot 6H_2O$, 0.04; $MnSO_4 \cdot H_2O$, 0.00025; trisodium citrate, 1; thiamine hydrochloride, 0.01; L-glutamic acid, 2; glycine, 0.2; vitamin-free acid-hydrolyzed casein (Nutritional Biochemicals Corp., Cleveland, Ohio), 5; glucose, 5. For solid media, 15 g of agar was added per liter.

Spores were grown on potato agar as described previously (6).

Recipient cells for transduction were grown in 250-ml Erlenmeyer flasks containing 25 ml of BHI-glycerol broth and were incubated at 37°C on a rotary shaker at 250 rpm. Cultures were grown for 4 to 5 h from a 10% (vol/vol) transfer of a 15-h culture or for 6 to 7 h from an inoculum of about 2×10^8 spores per flask.

Transductions. Two methods were routinely used in transductions. *B. cereus* and *B. thuringiensis*, for which phage CP-51 is rather lytic, were transduced in the following manner. A 1- to 2-ml volume of recipient cell culture containing 4×10^8 to 8×10^8 cells per ml was mixed in a 20-mm tube with 0.5 to 1 ml of phage suspension and incubated on a shaker at 37°C. After 1 h, 0.1 ml of phage antiserum was added, and incubation was continued for an additional 1 to 2 h to allow phenotypic expression of antibiotic resistance. Samples (0.1 ml each) were spread together with 0.1 ml of phage antiserum on L-agar supplemented with 25 μg of the appropriate antibiotic per ml. Plates were incubated at 37°C, and transductants were scored after 36 h.

Transductions of *B. anthracis*, for which phage CP-51 is less lytic, were performed as follows. Recipient cells, prepared as above, and phage (0.1 ml of each) were spread together on Millipore HA membranes (Millipore Corp., Bedford, Mass.) placed on L-agar plates. The plates were incubated at 37°C for 4 to 5 h, after which time the membranes were transferred to L-agar supplemented with 25 μg of the appropriate antibiotic per ml. Incubation was continued for 36 h.

Phage to be used in tranductions was sometimes treated with UV light to inactivate 95 to 99% of the PFU (6), and the multiplicity of infection (MOI) was calculated on the basis of titer before UV treatment. Tests for spontaneous antibiotic resistance by substituting NBY broth for the phage were always included. Representative transductants were tested for retention of their auxotrophic or streptomycin resistance

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TABLE 1. Strains used in this study

Strain	Characteristics[a]	Origin/reference
B. anthracis		
Weybridge	Nonvirulent	MRE[b]
Weybridge A	Colonial variant of Weybridge	C. B. Thorne
Weybridge A UM18	*pyrA*	UV[c] of Weybridge A
Weybridge UM44	*trp-1*	UV of Weybridge
B. cereus		
GP7	Carries pBC16, Tc[r]	W. Goebel
569	Wild type	NRRL[d]
569 UM47	*pur-1*	UV of 569
B. thuringiensis		
4042		NRRL[e]
4042A	*B. thuringiensis* subsp. *thuringiensis*	From 4042[e]
4042B	*B. thuringiensis* subsp. *aizawai*	From 4042[e]
4042B UM45	*trp-1*	UV of 4042B
4043	*B. thuringiensis* subsp. *dendrolimus*	NRRL
4049	*B. thuringiensis* subsp. *morrisoni*	NRRL
4050	*B. thuringiensis* subsp. *tolworthi*	NRRL
4059	*B. thuringiensis* subsp. *toumanoffi*	NRRL
4060	*B. thuringiensis* subsp. *thompsoni*	NRRL
4060C	Colonial variant of 4060	C. B. Thorne
4060C UM473	*trp-17*	UV of 4060C
YAL	*B. thuringiensis* subsp. *alesti*	A. Yousten
YGA	*B. thuringiensis* subsp. *galleriae*	A. Yousten
YGA UM1	*str-1*	Spontaneous from YGA
BIS	*B. thuringiensis* subsp. *israelensis*	H. deBarjac
HD1	*B. thuringiensis* subsp. *kurstaki*	A. Yousten
4D11A	Carries pC194, Cm[r]	D. Dean

[a] Abbreviations: Tc[r], plasmid-encoded tetracycline resistance; Cm[r], plasmid-encoded chloramphenicol resistance.

[b] MRE, Microbiological Research Establishment, Porton, England.

[c] Mutagenesis by UV light.

[d] NRRL, Agricultural Research Service, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill.

[e] Three serotypes corresponding to *B. thuringiensis* subsp. *thuringiensis*, *B. thuringiensis* subsp. *aizawai*, and *B. thuringiensis* subsp. *sotto* were isolated from NRRL 4042.

marker by replica plating colonies to Min 3C agar with and without the appropriate supplement.

Phage antiserum. Antiserum to phage CP-51 was prepared in rabbits by intravenous injection of phage suspensions having 10^{10} or more PFU per ml (6).

Plasmid extraction. Transductants were screened for plasmid DNA by the methods of White and Nester (11) and Kado and Liu (5) except that protoplasts were used in place of cells. For production of protoplasts, cells were grown in 250-ml Erlenmeyer flasks containing 25 ml of BHI-glycerol broth and were incubated at 37°C on a rotary shaker. Cultures were grown for 3 h from a 0.2-ml inoculum of a 15-h L-broth culture. Penicillin G (10 μg/ml) was added for the final 15 min of growth. Cells were collected by centrifugation at 10,000 rpm in a Sorvall SS34 rotor for 15 min at 4°C and suspended in a solution (pH 7.5) containing the following (in grams per liter): $(NH_4)_2SO_4$, 2; KH_2PO_4, 6; K_2HPO_4, 14; trisodium citrate, 1; $MgSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.00025; L-glutamic acid, 2; sucrose, 200; lysozyme, 5. The cells were converted to protoplasts at 37°C during incubation on a rotary shaker for 2 h. They were harvested by centrifugation at 6,000 rpm for 20 min at 4°C and suspended in 0.5 ml of 40% sucrose.

RESULTS

Transduction of pBC16. Phage CP-51 propagated on *B. cereus* GP7 was used to examine the transfer of plasmid-encoded tetracycline resistance to *B. anthracis*, *B. cereus*, and *B. thuringiensis* by transduction. Table 2 shows the results of pBC16 transduction from GP7 to tetracycline-sensitive strains of these three species. Phage grown on GP7 transferred tetracycline resistance to all three species at frequencies which were more than sufficient to recover transductants. In addition, Table 2 shows that the recipients of pBC16 served effectively as donors of tetracycline resistance in transductions both within and between species. DNA isolated from representative transductants was analyzed by gel electrophoresis for the presence of the plasmid. Figure 1 shows that tetracycline-resistant transductants contained newly acquired plasmid DNA with a mobility indistinguishable from that of pBC16 isolated from GP7. The antibiotic resistance of transductants was always associated with the acquisition of plasmid DNA. The transductants retained the auxotrophic or streptomycin resistance marker of the recipient strain. *B. thuringiensis* 4060C and *B. cereus* 569 exhibited low frequencies of spontaneous mutation to tetracycline resistance. However, the spontaneous mutants grew much more slowly than the transductants and produced smaller colonies. Thus, they were easily distinguished from transductants. At the time transductants were scored, the numbers of spontaneous tetracycline-resistant mutants were negligible.

In addition to the strains shown in Table 2, pBC16 was transduced into all other strains of *B. thuringiensis* tested, including *B. thuringiensis* subsp. *israelensis*, *B. thuringiensis* subsp. *alesti*, *B. thuringiensis* subsp. *kurstaki*, *B. thuringiensis* subsp. *dendrolimus*, *B. thuringiensis* subsp. *morrisoni*, *B. thuringiensis* subsp. *tolworthi*, and *B. thuringiensis* subsp. *toumanoffi*. The frequency of plasmid transduction was significantly affected by the MOI. Transductions in which the phage-to-cell ratio was less than 1 were among those giving the highest transduction frequencies. The transduction of pBC16 from *B. anthracis* to *B. thuringiensis* 4042B UM45 (Table 2) very strikingly illustrates this effect.

Transduction of pC194. Phage CP-51 was propagated on *B. thuringiensis* 4D11A to test for the transfer of pC194 by transduction. Results demonstrating transduction of pC194 to *B. anthracis*, *B. cereus*, and *B. thuringiensis* are shown in Table 3. The frequency of transfer was high enough to allow easy recovery of the transductants. The transductants of *B. cereus* 569 carrying pC194 served effectively as donors of the plasmid. Representative transductants were tested for the presence of pC194 by analyzing cell lysates by gel electro-

TABLE 2. Transduction of plasmid pBC16

Donor	Recipient	MOI	Tc^r colonies per:	
			ml	PFU
B. cereus GP7(pBC16)	*B. thuringiensis* 4042B UM45	9[a]	35	1.0×10^{-8}
	B. thuringiensis 4060C UM473	9[a]	5	1.4×10^{-9}
	B. anthracis Weybridge A UM18	9[a]	5	1.4×10^{-9}
	B. cereus 569 UM47	7	100	1.2×10^{-7}
B. thuringiensis 4042B UM45(pBC16)	*B. thuringiensis* 4042B UM45	13[a]	228	1.4×10^{-8}
	B. thuringiensis 4060C UM473	13[a]	107	6.6×10^{-9}
	B. anthracis Weybridge UM44	85	500	9.8×10^{-9}
	B. cereus 569 UM47	85	600	1.2×10^{-8}
B. thuringiensis 4060C UM473(pBC16)	*B. thuringiensis* 4042B UM45	13[a]	253	1.6×10^{-8}
	B. thuringiensis 4060C UM473	13[a]	2,186	1.4×10^{-7}
	B. anthracis Weybridge A UM18	175[a]	200	2.8×10^{-9}
B. anthracis Weybridge A UM18(pBC16)	*B. thuringiensis* 4042B UM45	8	295	6.1×10^{-8}
		0.6	4,900	1.3×10^{-5}
	B. thuringiensis 4060C UM473	8	75	1.6×10^{-8}
	B. thuringiensis YGA UM1	0.6	6,210	1.7×10^{-5}
	B. anthracis Weybridge A UM18	8	5	1.0×10^{-9}
	B. cereus 569 UM47	0.6	2,100	5.7×10^{-6}
	B. cereus 569 UM47(pC194)	2[a]	130	1.0×10^{-8}
B. cereus 569 UM47(pBC16, pC194)	*B. anthracis* Weybridge A UM18	2	60	5.5×10^{-8}
		0.2	30	2.7×10^{-7}
		0.02	10	9.0×10^{-7}
	B. thuringiensis YGA UM1	4	2,100	9.8×10^{-7}
		0.4	1,150	5.0×10^{-6}
	B. thuringiensis 4042B UM45	0.4	876	3.8×10^{-6}
	B. cereus 569 UM47	0.4	1,236	5.4×10^{-6}

[a] Phage was treated with UV light to inactivate 97% of the PFU.

phoresis for plasmid DNA. The chloramphenicol-resistant transductants contained newly acquired plasmid DNA with a mobility indistinguishable from that of pC194 isolated from strain 4D11A (data not shown). Chloramphenicol resistance of transductants was always associated with the acquisition of plasmid DNA. Spontaneous mutants resistant to the antibiotic were not observed among the recipients used in transduction. All chloramphenicol-resistant transductants tested retained the auxotrophic or streptomycin resistance marker of the recipient strain.

Tests for cotransduction of pBC16 and pC194. Phage CP-51 propagated on *B. cereus* 569 UM47(pBC16, pC194) was used to test for the simultaneous transfer of pBC16 and pC194 to several strains. Cotransduction of the two plasmids could not be demonstrated. The transfer of tetracycline resistance in these transductions was observed at high frequencies (Table 2). However, none of the transductants could grow when they were subsequently tested for chloramphenicol resistance by being replica plated onto medium containing the antibiotic. Likewise, the transfer of chloramphenicol resistance was detected at high frequencies (Table 3), but none of these transductants was able to grow on medium containing tetracycline. No transductants were found when primary selection was on medium containing both antibiotics.

DISCUSSION

The results presented here demonstrate that phage CP-51 carries out interspecies transduction of plasmids pBC16 and pC194 among a large number of *B. thuringiensis* subspecies, *B. cereus* 569, and the nonvirulent Weybridge (Sterne) strain of *B. anthracis*. The plasmid-transducing ability of CP-51 was independent of the host on which the phage was grown. All strains carrying the transferred plasmids were able to serve as donors.

The frequencies of plasmid transductions were often high-

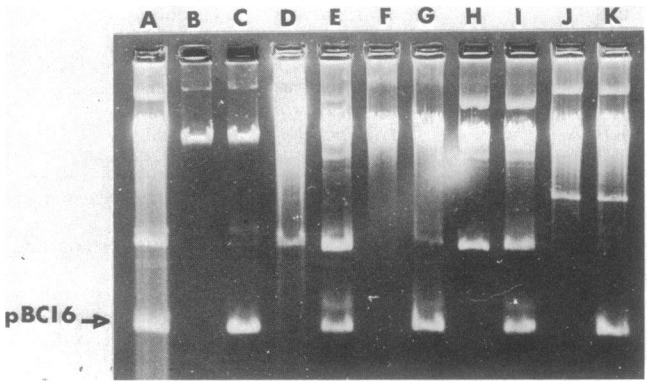

FIG. 1. Agarose gel electrophoresis of plasmid DNA from donors and recipients of pBC16. (A) *B. cereus* GP7; (B) *B. anthracis* Weybridge A UM18; (C) *B. anthracis* Weybridge A UM18(pBC16); (D) *B. thuringiensis* 4042B UM45; (E) *B. thuringiensis* 4042B UM45(pBC16); (F) *B. thuringiensis* 4060C UM473; (G) *B. thuringiensis* 4060C UM473(pBC16); (H) *B. thuringiensis* YGA UM1; (I) *B. thuringiensis* YGA UM1(pBC16); (J) *B. cereus* 569 UM47; (K) *B. cereus* 569 UM47(pBC16). For this photograph plasmid extracts prepared by the two referenced methods were combined. The method of White and Nester gave better resolution of small plasmids, and the methods of Kado and Liu gave better resolution of large plasmids.

TABLE 3. Transduction of plasmid pC194

Donor	Recipient	MOI	Cm^r colonies per:	
			ml	PFU
B. thuringiensis 4D11A(pC194)	*B. thuringiensis* 4042B UM45	40	140	5.8×10^{-9}
	B. cereus 569 UM47	40	130	5.4×10^{-9}
	B. anthracis Weybridge UM44	40	100	4.1×10^{-9}
B. cereus 569 UM47(pBC16, pC194)	*B. anthracis* Weybridge A UM18	2	10	9.0×10^{-10}
		0.2	20	2.0×10^{-8}
		0.02	80	7.3×10^{-7}
	B. thuringiensis 4042B	0.6	640	2.7×10^{-6}
		0.06	90	3.9×10^{-6}
	B. thuringiensis 4042A	0.6	50	2.1×10^{-7}
	B. thuringiensis YGA UM1	0.6	390	1.7×10^{-6}
		0.06	30	1.3×10^{-6}

er than those seen in the transduction of chromosomal markers. This may reflect the high copy number of the plasmids tested. Generally the factors employed to increase the yield of chromosomal transductants also increased the yield of plasmid transductants. These factors were (i) a low MOI, (ii) the treatment of transduction mixtures with phage antiserum, and (iii) plating on a rich medium. Unlike chromosomal transductions, plasmid transductions did not increase in frequency after the inactivation of a large proportion of PFU with UV light. Most likely this outcome resulted from the relatively large target size of the UV-sensitive replicons compared with the small chromosomal segment which must recombine with the recipient chromosome (1).

Our failure to detect cotransduction of pBC16 and pC194 may indicate that phage CP-51 can carry only a single piece of DNA. If this is true, cotransduction would require a plasmid cointegration event. Cointegration frequencies may have been too low to permit the detection of cotransduction in our experiments.

By using phage CP-51-mediated transduction, we have placed antibiotic resistance plasmids into strains of three related species which originally contained only unselectable or cryptic plasmids. This allows us to monitor plasmid movement in experiments which test for cocuring or cotransfer during transformation, protoplast fusion, transduction, or conjugation. We have found that the transduction method is more reproducible than currently published transformation procedures for strains of these species. Plasmids constructed in vitro or those carried by strains outside the host range of CP-51 must be introduced initially into CP-51-sensitive strains by transformation. Since many strains of *B. cereus*, *B. anthracis*, and *B. thuringiensis* may be refractile to plasmid transformation, plasmid transduction from an intermediate host which is transformable may be useful.

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